Journal of Medicinal Plants Research Vol. 5(32), pp. 7031-7035, 30 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.1159

Full Length Research Paper

Biological and phytochemical studies on corms of *Colchicum luteum* Baker

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Accepted 20 September, 2011

The methanol extract of the corms of the *Colchicum luteum* Baker (Liliaceae) and its subsequent solvent fractions were screened for brine shrimp cytotoxic, phytotoxic, insecticidal activities and phytochemical studies. Profound cytotoxicity was displayed by the crude methanolic extract (LD_{50} 42.43 µg/ml). However, the cytotoxic potential was not much altered by the fractionation. The plant also expressed low phytotoxicity against *Lemna acquinoctialis* Welv and the highest phytotoxicity was exhibited by the ethyl acetate fraction (33%) at 1000 µg/ml. Interestingly, negative phytotoxic effect was also computed; aqueous fraction expressed maximum phytotoxic effect (13.79%) at 10 µg/ml. Overall poor insecticidal activity was observed in which the *n*-butanol fraction exhibited the highest activity against *Callosdruchus analis* (40%) followed by the chloroform fraction (35%). Results revealed significant cytotoxicity of the extracts and therefore, can be a potential new natural source for the treatment of different types of cancers. Phytochemical studies showed the presence of various pharmacological groups especially alkaloids, phenols, flavonoids and saponins.

Key words: *Colchicum luteum*, cytotoxicity, phytotoxicity, insecticidal.

INTRODUCTION

Colchicum luteum Baker commonly known as Suranjan-e-Talkh (Urdu) and belongs to family Liliaceae (Shinwari et al., 2003). The Liliaceae represent mostly perennial herbs from starchy rhizomes, corms, or bulbs comprising about 280 genera and 4,000 species. The leaves are alternate or less often opposite or whorled. The flowers are nearly always bisexual and actinomorphic. The genus colchicum includes 42 species, most of which are endemic to Middle East and South Africa to Western Europe and Asia (Brickell, 1984; Sahibzada et al, 2006). Corms of the *C. luteum* Baker are extensively used for the treatment of gout, rheumatism and diseases of the liver and spleen. The corms are also used as blood purifier (Bashir et al., 2006a). The poisonous alkaloid colchicine is extracted from *C. luteum* (Bashir et al., 2006b), is used for the treatment of Behçet's syndrome (Wechsler, 2002) and a topical remedy for penile condylomata acuminata (Von, 1978, 1981). Crude methanol extract and subsequent fractions of the *C. luteum* Baker expressed outstanding inhibition on lipoxygenase and significant antimicrobial activity (Bashir et al., 2006a; Bashir et al., 2006b). Phytochemically, the mainstay of the genus, colchicum is alkaloids including *C. luteum* (Bashir et al., 2006a). From the colchicum, thirty-one different alkaloids have been isolated (Capraro and Brossi, 1984). Colchicine is the main alkaloid isolated from all species of the genera, colchicum (Peter, 1995). In the current study, we present investigations on the brine-shrimp cytotoxicity, phytotoxicity, insecticidal activities and detailed phytochemical studies of the crude methanolic extract and subsequent solvent fractions.

MATERIALS AND METHODS

Plant material

The *C. luteum* Baker, as a whole plant was collected from Sherengel, Upper Dir, Khyber Pukhtonkhawa (Pakistan) during

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February to March 2004. The plant material was identified by Prof. Dr. Jahandar Shah, plant taxonomist and verified by Dr Abd-ur-Rashid, Department of Botany, University of Peshawar.

Extraction

The shade dried plant material was chopped into small pieces and finally pulverized into fine powder. The powdered plant material (10 kg) was macerate in methanol (3 × 10 L), with occasional shaking, at room temperature.

After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator (Khan et al., 2010). A yellowish crude extract (246 g) was obtained.

Fractionation

The crude methanolic extract (246 g) was suspended in distilled water (500 ml) and sequentially partitioned with *n*-hexane (3 × 500 ml), chloroform (3 × 500 ml), ethyl acetate (3 × 500 ml) and *n*-butanol (3 × 500 ml) to yield the *n*- hexane (26 g), chloroform (59 g), ethyl acetate (32 g), *n*-butanol (35 g) and aqueous (68 g) fractions, respectively.

Brine-shrimp cytotoxicity

Brine-shrimp eggs (*Artemia salina*) were used to determine the cytotoxic effect of the crude extract and subsequent fractions of *C. luteum* (Saeed et al., 2010a). In artificial sea- water, the shrimps were allowed for 48 h at room temperature (22 to 29°C) to hatch and mature. Prepared vials for testing of each fraction, tested initially at 1000, 100 and 10 µg/ml. Prepared triplicate for each concentration making a total of 9 vials. Then weighed 20 mg of sample and added 2 ml of organic solvent (20 mg/2 ml). From this, solution was transferred to 500, 50 or 5 vials corresponding to 1000, 100 or 10 µg/ml respectively. Evaporated solvents under nitrogen and then placed under high vacuum for about 30 min. The volatile organic solvents evaporated overnight. After 2 days, when the shrimp larvae had matured, sea-water (5 ml) was added to each vial containing 10 shrimps per vial (30 shrimps per dilution). The vials were maintained under illumination. After 24 h, the number of the surviving shrimps was counted with the help of a 3x magnifying glass. The data Analyzed using a Finny computer programmed (probit analysis) in order to determine LD_{50} values with 95% confidence intervals.

Phytotoxic assay

Phytotoxic activity of the extracts was carried against *Lemna acquinoctialis* Welv (Saeed et al., 2010b). The medium was prepared by mixing various inorganic constituents in distilled water (100 ml) and the pH was adjusted (5.5 to 6.5) by adding KOH solution.

The medium was then autoclaved at 121°C for 15 min. The samples (30.0 g) dissolved in ethanol (15 ml) served as stock solution. Sterilized 9 flasks, three for each concentration, were inoculated with 1000, 100 and 10 µl of the stock solution to give the final concentration of 1000, 100 and 10 µg/ ml respectively. The solvent was allowed to evaporate overnight under sterile condition. To each flask, 20 ml of the medium at a pH of 5.5 to 6.5 was added. Then 10 plants of *L. acquinoctialis* Welv, each containing a rosette of three fronds was added to each flask. One other flask was supplemented with solvent, and reference plant growth inhibitor (paraquat) that served as negative control. All flasks were plugged with cotton and kept in the growth cabinet for 7 days. The number of fronds per flask were counted and recorded on day seven.

% Growth inhibition = 100 - No. of frond in test / No. of frond in control × 100

Insecticidal assay

The crude extract and its subsequent fractions were screened against various insects viz., *Rhyzopertha dominica, Tribolium castaneum* and *Callosobruchus analis* (Saeed et al., 2010b). Briefly, the filter papers were cut according to the size of Petri plates (9 cm or 90 mm) and put them in to plates. Samples were loaded over the papers with the help of micropipette and left for 24 h for solvent evaporation. Next day, 10 insects of each species was put in each plate (test and control), incubated the plates at 27°C for 24 h with 50% relative humidity in growth chamber. The number of survival of each species was counted and % mortality was computed.

% Mortality = 100 - No. of insect alive in test / No. of insect alive in control × 100.

Phytochemical test

Preliminary phytochemical tests were carried out according to the previously described methods (Nisar et al., 2008; Kumar et al., 2011). For the detection of alkaloids, 0.2 g of the extract was warmed with 2% H_2S0_4 for two min, filtered and few drops of Draggendorff's reagent was added. Orange red precipitate indicates the presence of alkaloids. For saponins, approximately 0.2 g of the sample was shaken with 5 ml of distilled water. The appearance of frothing on boiling indicates the presence of saponins. 1 g of extract was mixed with 2 ml of distilled water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate.

A dark green solution indicates the presence of tannins. 0.2 g of test sample was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids. About 0.5 g of the each extract was boiled with 10% HCl for few min on a water-bath, filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 was added to the mixture and heated. Formation of rose-pink colour indicates the presence of Anthraquinone. For terpenoids (Salkowski test), 0.2 g of sample was mixed with 2 ml of chloroform. To this, 3 ml of concentrated H_2S0_4 was carefully added to form a layer. The presence of terpenoids was demonstrated by the formation of reddish brown colour on the interface.

RESULTS AND DISCUSSION

Effect of brine-shrimp cytotoxic assay

Brine-shrimp eggs (*A. salina*) were used to determine cytotoxicity. A significant cytotoxic activity was computed for the crude methanolic extract and subsequent fractions of *C. luteum* Baker and results are presented in Table 1. Crude extract expressed significant cytotoxic activity against brine-shrimp and median lethal dose (LD_{50}) was measured as 42.43 µg/ml. Upon sequential fractionation the toxic potential of the *C. luteum* Baker was not much

Table 1. Brine shrimp cytotoxicity of the extracts of *Colchicum luteum* Baker.

Tests material	Extracts	LD_{50} (µg/ml)
Artemia salina	Crude extract	42.43
	Chloroform	42.43
	Ethyl acetate	43.30
	n-Butanol	42.43
	Aqueous	45.15
	Standard (Etoposide)	07.4625

Incubation at 28±1°C.

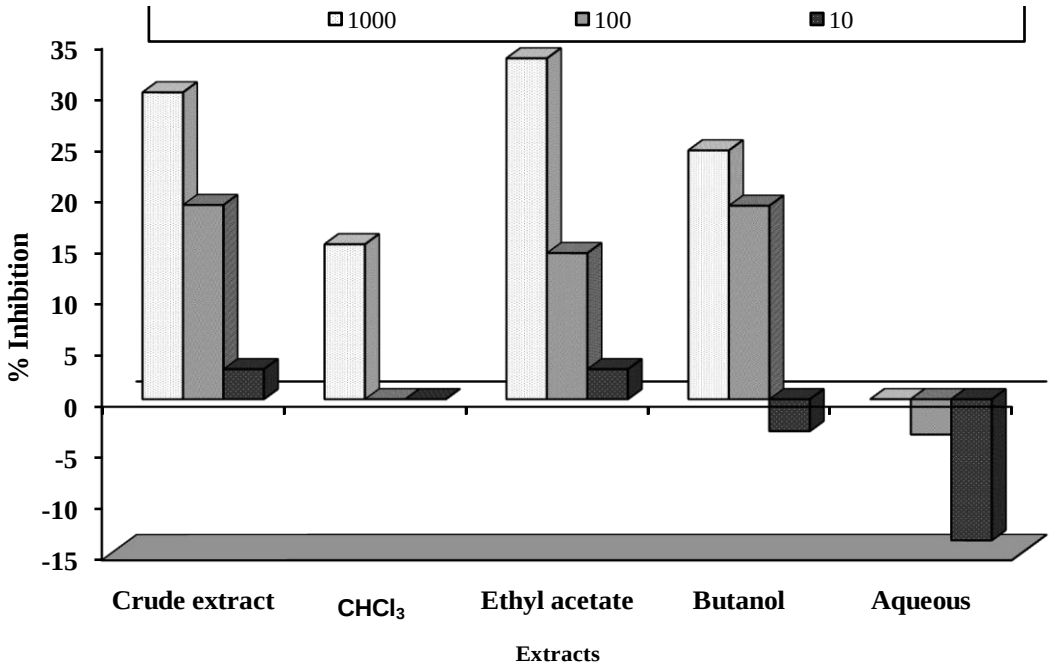

Figure 1. Phytoxic activity of the extracts of *Colchicum luteum* Baker against *Lemna acquinoctialis* Welv.

changed and the chloroform fraction and *n*-butanol fraction expressed the same cytotoxicity ($LD_{50:}$ 42.43 µg/ml). The cytotoxic trend was declined a little in the case of ethyl acetate fraction and LD_{50} was observed as 43.30 µg/ml. The aqueous fraction demonstrated the least cytotoxic activity with LD_{50} 45.15 µg/ml in the assay.

Effect of phytotoxic activity

Phytotoxic activity of the crude extract and various fractions of *C. luteum* Baker were carried against the *L. acquinoctialis* Welv. The results of phytotoxic activities are shown in Table 2 and Figure 1. The crude extract of the *C. luteum* Baker showed (30%) activity at 1000, (19%) at 100 and about 3% at 10 µg/ml, while no negative effect on plant growth. Chloroform fraction showed low phytotoxic activity at 1000 µg/ml concentration, while no activity at 100 and 10 µg/ml concentrations. In case of ethyl acetate fraction of *C. luteum*, the phytotoxic effect was 33.33, 14.286 and 2.941% at 1000, 100 and 10 µg/ml, respectively.

Interesting activities was displayed by the *n*-butanol fraction of *C. luteum* Baker at 10 µg/ml because it supported the growth of plant (*L. acquinoctialis* Welv) by (3.125%), while the rest of the concentrations posted low phytotoxic activity. Similarly, low concentrations of the aqueous fraction also exhibited negative phytotoxic effects and the highest negative phytotoxic activity was expressed at 10 (13.793%) and (3.448%) 100 µg/ml.

Effect of the insecticidal activity

The crude extract and various fractions of *C. luteum* Baker were screened against various insects like

Table 2. Phytotoxic activities of extracts of *Colchicum luteum* Baker.

Dose (µg/ml)	Number of fronds									
	Crude extract		Chloroform		Ethyl acetate		*n*-butanol		Aqueous	
	Cont	Sam	Cont	Sam	Cont	Sam	Cont	Sam	Cont	Sam
1000	28	40	28	33	28	42	28	37	28	28
100	30	37	30	30	30	35	30	37	30	29
10	33	34	33	33	33	34	33	32	33	29

Data represents mean of three different experiments. Cont = Control, Sam = Sample. Standard = Paraquat (0.902 µg/ml).

Table 3. Insecticidal activities of extracts of *Colchicum luteum* Baker.

Name of Insect	Std	Mortality (%)									
		Crude		Chloroform		EtOAc		*n*-BuOH		Aqueous	
		NC	Sam	NC	Sam	NC	Sam	NC	Sam	NC	Sam
Rhyzopertha dominica	100	-	35	-	-	-	-	-	33	-	-
Tribolium castaneium	100	-	-	-	25	-	-	-	-	-	-
Callosdruchus analis	100	-	15	-	35	-	-	-	40	-	-

Data represents mean of three different experiments. NC = negative control; Sam = Sample; EtOAc = Ethyl acetate; Std = Standard (Permethrin) = 235.71 µg/cm^2, 1571.33 µg/cm^2.

Table 4. Results of preliminary phytochemical tests.

Fractions	Alkaloid	Phenol	Flavonoids	Sterol	Triterpenoid	Tannin	Saponins
Methanolic	+	+	+	+	–	+	+
n-Hexane	–	–	–	+	–	–	–
$CHCl_3$	+	+	–	+	–	+	+
EtOAc	+	+	+	+	–	+	+
Butanol	+	+	+	–	–	+	+
Aqueous	+	+	+	–	–	+	+

R. dominica, Callosdruchus analis, and results are shown in Table 3. The crude extract showed little activity against *R. dominica* (25%) and *C. analis* (15%). The chloroform fraction showed low activity against *C. analis* (25%) and *C. analis* (35%). In case of *n*-butanol fraction, it showed 33% for *R. dominica* and against *C. analis* (44%). The rest of fraction were displayed no activities against these insects.

Effect of phytochemical tests

The corm of the plant showed the presence of various chemical groups (Table 4).

Based on the available literature, fatal intoxication by the ingestion of colchicum species is rare and only few cases are reported (Klintschar et al., 1999; Sannohe et al., 2002). However, colchicine and other colchiconoid alkaloids are the foremost active ingredients isolated from this plant are responsible for the toxicity (Jayaprakash et al., 2007). Thus, it should be carefully used in the indigenous system of treatment to avoid any unwanted effect of the plant. On the other hand, cytotoxic compounds from this plant could play an important role in the treatment of various cancers. Therefore, in the course of new drug development, this plant species need comprehensive toxicity studies for its safe use in the traditional system and phytochemical investigation to isolate clinically effective molecules for the treatment of various cancers.

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